

CLAIMS

1. Solid phase immunoassay comprising on said solid phase an antigen in the presence of a reducing agent.
2. Method for producing or carrying out an immunoassay according to claim 1, wherein said reducing agent is added to said solid phase during the steps of coating, blocking and/or fixation of said antigen to said solid phase or during pretreatment of the solid phase.
3. Method according to claim 2 wherein said reducing agent is added to said solid phase during the step of coating the antigen to the solid phase.
4. Method according to claim 2, wherein said reducing agent is added to said solid phase during the step of blocking said solid phase comprising the antigen applied thereto in the presence or absence of a reducing agent.
5. Method according to claim 2, wherein said reducing agent is added to said solid phase during the step of fixation of said solid phase comprising the antigen applied thereto in the presence or absence of a reducing agent.
6. Method according to claim 2, wherein said reducing agent is added during pretreatment of the solid phase comprising the antigen applied thereto in the presence or absence of a reducing agent.
- Claim 1

7. Method according to any of claims 1 to 6 wherein said reducing agent is DTT, DTE or TCEP.

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claim 1 40

8. Method according to any of claims 1 to 7 wherein said reducing agent is used in a concentration range of 0.1 mM to 1 M, more particularly from 0.5 mM to 500 mM, even more particular from 1 mM to 250 mM, some applications may require ranges from 0.5 to 50 mM, 1 to 30 mM, 2 to 20 mM, or 5 to 15 mM, or about 10 mM.

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Claim 2

9. Method according to any of claims 2 to 8 wherein said antigen is an HCV NS3 protein.

C 5 10. Solid phase immunoassay produced by a method according to any of claims 2 to 9.

C 11. ELISA produced by a method according to any of claims 2 to 9.

12. ELISA according to claim 11 wherein said reducing agent is added in the coating and/or fixation steps.

13. QUICK test produced by a method according to any of claims 2 to 9.

10 14. QUICK test according to claim 13 wherein said reducing agent is added in the blocking step.

15. Line Immunoassay produced by a method according to any of claims 2 to 9.

16. Line Immunoassay according to claim 15 wherein said reducing agent is added in the blocking step.

Sub A! 15 17. Use of an assay according to claims 10 to 16 for in vitro diagnosis of antibodies raised against an antigen as described in claim 1.

18. HCV NS3 protein treated by a method comprising the steps of sulphonation and subsequent desulphonation.

19. HCV NS3 protein according to claim 18 which is additionally treated with a zwitter-ionic detergent, preferably Empigen.

20. Method for purifying a cysteine containing recombinantly expressed protein comprising at least 2, preferably 3 or 4 and even more preferably all of the following steps:

(a) sulphonation of a lysate from recombinant host cells or lysis of recombinant host cells in the presence of guanidinium chloride followed by a subsequent sulphonation of the cell lysate,

(b) treatment with a zwitterionic detergent, preferably after removal of the cell debris,

(c) purification of the sulphonated version of the recombinant protein or purification of the sulphonated version of the recombinant protein with subsequent removal of the zwitterionic detergent, with said purification being preferably chromatography, more preferably a Ni-IMAC chromatography with said recombinant protein being a His-tagged recombinant protein,

(d) desulphonation of the sulphonated version of the recombinant protein, preferably with a molar excess of DTT,

(e) storage in the presence of a molar excess of DTT, or immediate use in an assay.

21. An HCV polynucleic acid encoding a polypeptide as depicted in Figure 1 (SEQ ID NOS 3-18) or a unique part of an HCV polynucleic acid, more particularly a polynucleic acid having a sequence as represented in Figures 2-1, 3-1, 4-1, 5-1, 6-1, 7-1 or 8-1 (SEQ ID NOS 19, 21, 23, 25, 27, 29 and 31).

22. An HCV polynucleic acid according to claim 21 as depicted in Figures 2-1, 3-1, 4-1, 5-1, 6-1, 7-1 or 8-1 and characterized by the fact that its product does not react with falsely positive

HCV samples, or a part thereof which encodes a NS3 epitope which does not react with falsely positive HCV samples.

a 23. A recombinant vector comprising a polynucleic acid according to claim 21 or ~~22~~.

a 24. A host cell comprising a vector according to claim 23.

a 25. A method for detecting a nucleic acid sequence according to claim 21 or 22 comprising:

5 -contacting said nucleic acid with a probe
-determining the complex formed between said nucleic acid and said probe.

C 26. An isolated nucleic acid according to claim 21 or 22 or a fragment thereof for use as a probe or a primer for the detection of a nucleic acid according to claim 21 or 22.

a 10 27. A diagnostic kit for the detection of a nucleic acid sequence according to claim 21 or 22, comprising at least one primer and/or at least one probe according to claim 26.

a 28. An HCV polypeptide having part or all of the amino acid sequences of a polypeptide encoded by a polynucleic acid according to claim 21 or 22.

15 29. An HCV NS3 helicase protein or part thereof containing either S1200, A1218, A1384, P1407, V1412, P1424, or F1444, or a combination of these amino acids with any of the following amino acids L1201, S1222, I1274, S1289, T1321, A1323, T1369, L1382, V1408, A1409, F1410.

C 30. A pharmaceutical composition comprising a polypeptide according to claim 28 or 29, or

any functionally equivalent variant or fragment thereof.

a 31. A pharmaceutical composition comprising a polypeptide according to claim 28 or ~~29~~, or any functionally equivalent variant or fragment thereof for use as a medicament to prevent or treat HCV infection.

a 32. A method for detecting a polypeptide according to claim 28 or ~~29~~ comprising:

5 -contacting said polypeptides with a ligand binding to said polypeptide

-determining the complex formed between said polypeptide and said ligand.

a 33. A ligand binding to a polypeptide according to claim 28 or ~~29~~.

34. A composition comprising at least a ligand according to claim 33, in a pharmaceutical acceptable excipient, for use as a medicament.

a 10 35. A method for the production of a polypeptide according to claim 28 or ~~29~~ for diagnostic or therapeutic purposes.

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ABSTRACT